

ABSTRACT

The invention provides peptide synthons having protected functional groups for attachment of desired moieties (e.g. functional molecules or probes). Also provided are peptide conjugates prepared from such synthons, and synthon and conjugate preparation methods including procedures for identifying the optimum probe attachment site. Biosensors are provided having environmentally sensitive dyes that can locate specific biomolecules within living cells and detect chemical and physiological changes in those biomolecules as the living cell is moving, metabolizing and reacting to its environment.

5 Methods are included for detecting GTP activation of a Rho GTPase protein using polypeptide biosensors. When the biosensor binds GTP-activated Rho GTPase protein, the environmentally sensitive dye emits a signal of a different lifetime, intensity or wavelength than when not bound. New fluorophores whose fluorescence responds to environmental changes are also provided that have

10 improved detection and attachment properties, and that can be used in living cells, or *in vitro*.

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